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Three Novel TBX5 Mutations in Chinese Patients With Holt-Oram Syndrome

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Holt-Oram syndrome (HOS) is an autosomal dominant syndrome that comprises upper limb and cardiac defects. The gene responsible for HOS, *TBX5*, was isolated and many mutations have been identified in HOS patients. We analyzed 11 Chinese HOS patients (7 from three families and 4 sporadic cases) for *TBX5* mutation by single strand conformation polymorphisms (SSCPs). Three SSCP changes were detected in two of the three familial cases and one sporadic case. Sequence analysis identified three novel, heterozygous mutations in *TBX5*: a frameshift mutation caused by one base deletion [C416del] in one family, a mis-sense mutation (Gln49Lys) induced by a base substitution (C145A) in another family, and the other mis-sense mutation (Ile54Thr) by T161C in one sporadic case. The patients with the frameshift mutations had severer clinical manifestations that involved aplasia/hypoplasia of the arm and thumbs, while those with the mis-sense mutations presented with milder anomalies such as absent or hypoplastic thumbs but without arm abnormalities. These observations may support a genotype-phenotype correlation in HOS patients with *TBX5* mutation. Am. J. Med. Genet. 92:237–240, 2000.

KEY WORDS: gene mutation; TBX5; Holt-Oram syndrome; single strand conformation polymorphism

INTRODUCTION

Holt-Oram syndrome (HOS, MIM 142900) is an autosomal dominant developmental disorder with the prevalence of about 1/100,000 and characterized by congenital cardiac defects and upper limb malformations. The most common cardiac malformations in HOS are atrial septal defect (ASD), ventricular septal defect (VSD), and tetralogy of Fallot (TOF), while frequently observed upper limb anomalies include absent, short, branching, triphalangeal, and/or finger-like thumbs, and the absence of forearm, carpal, and/or radial bones [Poznanski et al., 1970]. Although the limb defects may be bilateral and asymmetrical, they often affect the left side predominantly. The HOS locus was assigned to chromosome 12q24.1 by linkage analysis [Terrett et al., 1994; Basson et al., 1994, 1997]. The causative gene for HOS, *TBX5*, was isolated from the 12q region by positional cloning [Li et al., 1997; Basson et al., 1997]. *TBX5* has homology to a member (*Tbx5*) of the mouse

TABLE I. Upper Limb and Cardiac Anomalies in Patients With Holt-Oram Syndrome

Patients	Upper limb anomaly	Heart anomaly
HOS-A1	Absent left arm and thumb; absent right forearm and thumb	ASD
HOS-A2	Curved radii and ulnae; absent thumbs	ASD
HOS-B1	Small thumbs; absent left middle finger; syndactyly between right middle and ring fingers	ASD
HOS-B2	Small left thumb	ASD
HOS-F1	Absent left thumb	ASD
HOS-F2	Curved radii and ulnae; absent thumbs	ASD
HOS-F3	Absent thumbs	ASD
HOS-C	Curved radii and ulnae; absent thumbs	ASD
HOS-D	Small left thumb	TOF; ASD
HOS-E	Absent left thumb	VSD
HOS-G	Absent left thumb	ASD

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TABLE II. Mutations in *TBX5* in Three Holt-Oram Syndrome Families

Family	Type of mutation	Exon	Position of mutation	Result of mutation
HOS-A	Deletion	4	C416del	Frameshift
HOS-B	Base substitution	2	C145A	Gln49Lys
HOS-G	Base substitution	2	T161C	Ile54Thr

T-box gene family, has an evolutionally conserved T-box (DNA binding) domain [Bollag et al., 1994], and acts as a transcription factor to adjust the growth and development process of embryos [Agulnik et al., 1995]. Gene expression studies on human embryos showed that *TBX5* plays a vitally essential role in the growth and development of the heart and upper limb [Li et al., 1997]. Although many *TBX5* mutations have been found in HOS patients [Basson et al., 1997, 1999; Li et al., 1997], no report exists on such mutations in Chinese patients.

This report deals with three novel mutations in *TBX5* observed in Chinese HOS patients.

MATERIALS AND METHODS

Patients and Their DNA Samples

We collected 11 Chinese HOS patients, including three familial (HOS-A, B, and F) and four sporadic (HOS-C, D, E, and G) cases. Family HOS-A included two patients (a mother, HOS-A1, and her son, HOS-A2), family HOS-B had two patients (HOS-B1 and HOS-B2), and family HOS-F contained three patients (HOS-F1, HOS-F2, and HOS-F3). Clinical manifestations in these 11 patients are shown in Table I. Blood samples were collected from the patients, their unaffected family members, and 50 normal control individuals. Genomic DNA was extracted from their leukocytes with the standard method.

PCR-SSCP Analysis and DNA Sequencing

TBX5 contains 8 exons, and its cDNA consists of 1,050 base pairs and encodes 349 amino acid [Basson et al., 1997]. We designed PCR-primer pairs from intronic sequences flanking exons 2–8 of *TBX5*. Sequence data were referred through GDB, accession number U80987. The primers were used to amplify the exonic sequences of HOS patients. PCR was performed for 30 cycles at 96°C for 15 sec, 58°C for 20 sec, and 72°C for 35 sec. After adding an equal amount of 2× denaturing carrier buffer solution, the products were denatured at 95°C for 5 to 10 min and quickly placed on ice for 5 to 10 min. Then, they were electrophoresed on non-denaturing polyacrylamide gel containing 5% glycerin, and the gels were dyed with silver nitrate. In addition, after the PCR products were purified, they were subjected to direct sequencing using an ABI 377 automatic DNA sequencer.

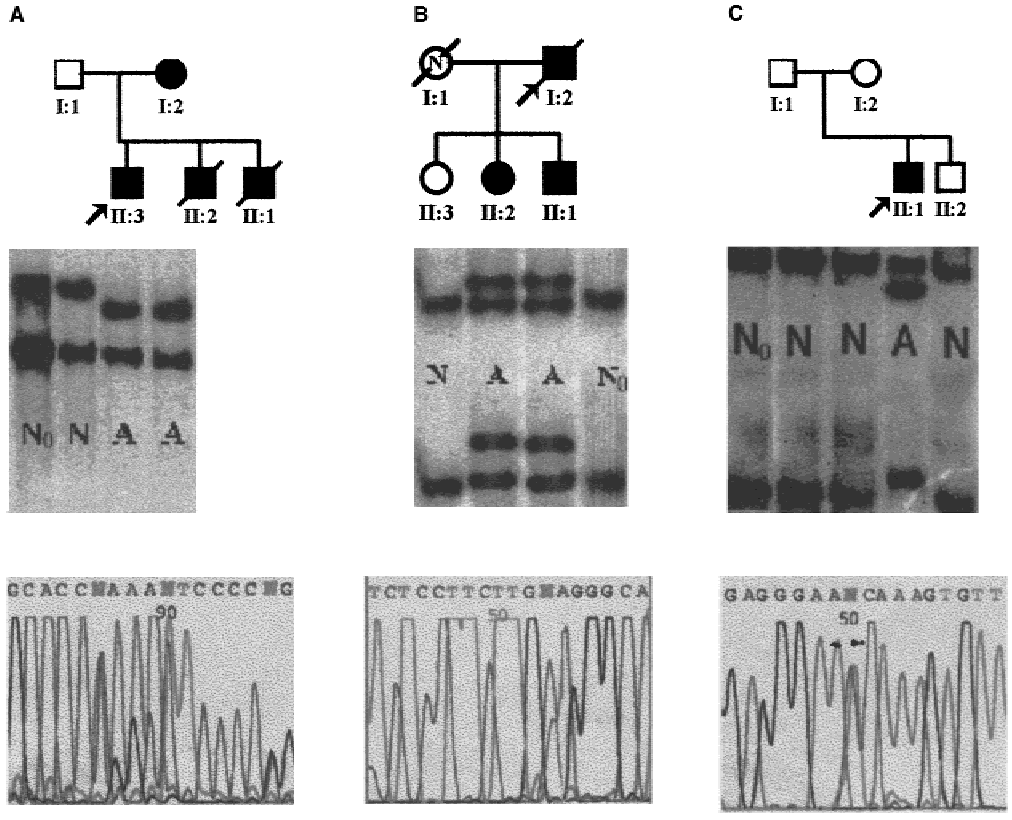

Fig. 1. Pedigrees (first row), SSCP patterns (second row) and base sequences of TBX5 (third row) in patients from families HOS-A (**A**), HOS-B (**B**), and from HOS-G (**C**). Arrows indicate probands.

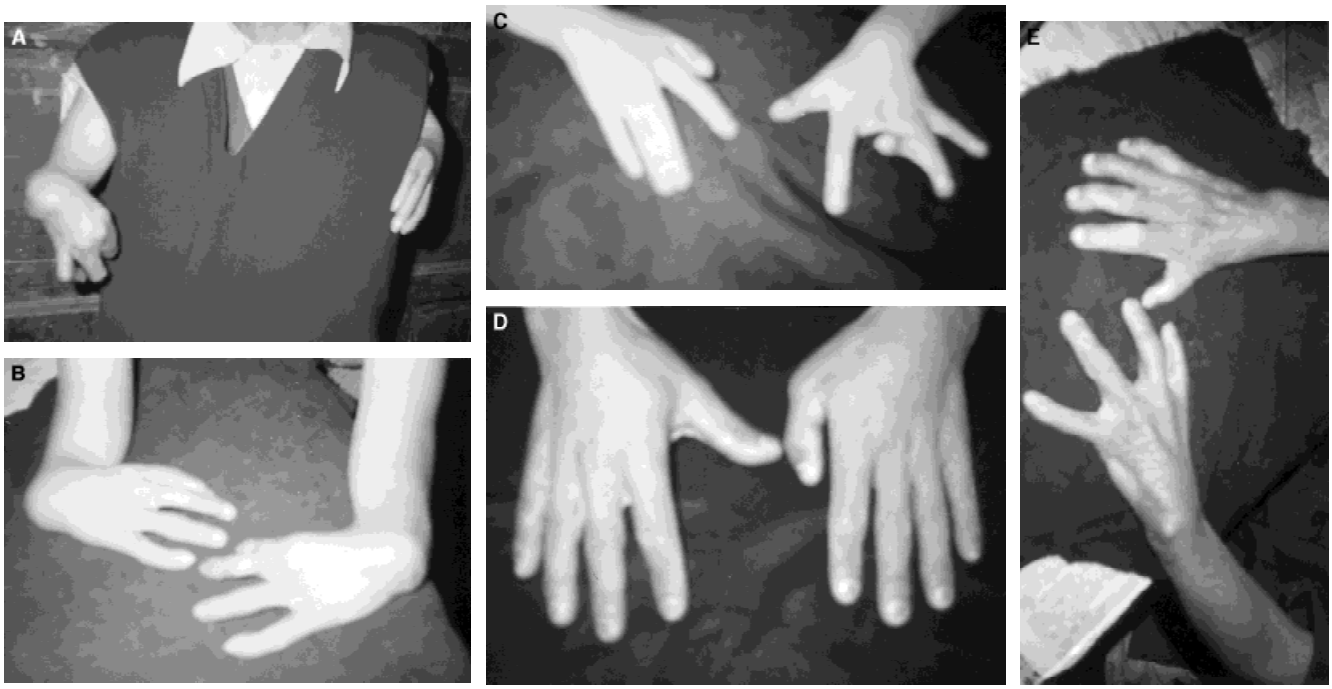

Fig. 2. Hand abnormalities in patients, HOS-A1 (**A**), HOS-A2 (**B**), HOS-B1 (**C**), HOS-B2 (**D**), and HOS-G (**E**).

RESULTS

We detected SSCP band alterations in the PCR products for exons 2 and 4 from three (HOS-A, HOS-B, and HOS-G) of the seven HOS families examined. Direct sequencing showed that both patients from family HOS-A had one base deletion at cDNA position 416 [C416del] (Table II, Fig. 1). Patients from family HOS-B showed a C → A substitution at cDNA position 145 (C145A). This mutation was never observed in any unaffected members of the family. The patient HOS-G has a T → C substitution at position 161 (T161C). The three alterations were all novel, heterozygous mutations, and none of them were identified in 100 chromosomes from 50 normal control individuals. In other familial and sporadic cases, no mutations in exons 2–8 of *TBX5* were identified.

DISCUSSION

We detected three novel mutations in *TBX5* in three of the seven HOS families examined. All mutations identified are located at either exon 2 or exon 4 of the gene (Table II). The one-base deletion [C416del] observed in affected individuals from family HOS-A causes frameshift of downstream codons. Since the C to A transversion occurring in family HOS-B predicts a substitution of glutamine (CAG) by lysine residue (AAG) at codon 49 (Gln49Lys), it may weaken the function of TBX5 protein, resulting in HOS. Likewise, the T161C nucleotide alteration in the patient HOS-G predicts a substitution of isoleucine (ATC) by threonine residue (ACC) at codon 54 (Ile54Thr), which may also reduce the protein function, leading to the syndrome. We were not able to detect mutations in *TBX5* exons 2–8 in the remaining four families. However, since HOS is causally heterogeneous [Basson et al., 1995], the failure of mutation search is not surprising. Alternatively, mutations could have been overlooked because they cannot always be identified by SSCP analysis. It remains to be investigated in these patients whether mutations exist in other part of *TBX5*, especially in 5′ or 3′ flanking region.

The severity of clinical manifestations in the patients may reflect the type of mutations. Two patients in family HOS-A with the frameshift mutation had severer clinical findings, such as absence of the left arm with a small left hand directly connected to the shoulder joint, absence of right forearm with a hand connected to the elbow joint, and absence of both thumbs. Her affected son also had maldevelopment of both upper limbs with curved radii and ulnae, and absence of thumbs. In contrast, the patients with Gln49Lys or Ile54Thr had absent or hypoplastic thumbs but no obvious abnormalities in their arms (Fig. 2), indicating milder clinical manifestations. These findings suggest that the severity of upper limb defects is closely associated with degree of loss of the *TBX5* function and support "genotype-phenotype correlation" suggested by Basson et al. [1999]. Basson et al. [1999] demonstrated that defects predicted to create null alleles cause substantial abnormalities in both limb and heart, while a mis-sense mutation such as Gly80Arg causes significant cardiac malformations but only minor skeletal abnormalities, and Arg237Gln and Arg237Trp leads to extensive upper limb malformations but less significant cardiac abnormalities. Further studies on more numbers of patients will be necessary to confirm the correlation.

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REFERENCES

Agulnik SI, Bollag RJ, Silver LM. 1995. Conservation of the T-box gene family from Mus musculus to caenorhabditis elegans. Genomics 25: 214–219.

Basson CT, Cowley GS, Solomon SD, Weissman B, Poznanski AK, Traill TA, Seidman JG, Seidman CE. 1994. The clinical and genetic spectrum of the Holt-Oram syndrome (heart-hand syndrome). N Engl J Med 330:885–891.

Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Soults J, Grayzel D, Kroumpouzou E, Traill TA, Leblanc-Stracesk J, Renault B, Kucherlapati R, Seidman JG, Seidman CE. 1997. Mutations in human cause limb and cardiac malformation in Holt-Oram syndrome. Nature Genet 15:30–35.

Basson CT, Huang T, Lin, RC, Bachinsky DR, Weremowicz S, Vaglio A, Bruzzone R, Quadrelli R, Lerone M, Romeo G, Silengo M, Pereira A, Krieger J, Mesquita SF, Kamisago M, Morton CC, Pierpont MEM, Muller CW, Seidman JG, Seidman CE. 1999. Different TBX5 interactions in heart and limb defined by Holt-Oram syndrome mutations. Proc Natl Acad Sci USA 96:2919–2924.

Bollag RJ, Siegfried Z, Cebra-Thomas JA, Garvey N, Davison EM, Silver LM. 1994. An ancient family of embryonically expresses mouse genes sharing a conserved protein motif with the T locus. Nature Genet 7: 383–389.

Li QY, Newbury-Ecob R, Terrett JA, Wilson DI, Curtis ARJ, Yi CH, Gebuhr T, Bullen PJ, Robson SC, Strachan T, Bonnet D, Lyonnet S, Young ID, Raeburn JA, Buckler AJ, Law DJ, Brook JD. 1997. Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachyury (T) gene family. Nature Genet 15:21–29.

Terrett JA, Newbury-Ecob R, Cross GS, Fenton I, Raeburn JA, Young ID, Brook JD. 1994. Holt-Oram syndrome is genetically heterogeneous disease with one locus mapping to human chromosome 12q. Nature Genet 6:401–404.

Poznanski AK, Gall JC Jr, Stern AM. 1970. Skeletal manifestations of the Holt-Oram syndrome. Radiology 94:45–54.